High efficiency delivery and expression of functional membrane proteins using Virapower™ HiPerform™ Lentiviral vectors

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Abstract
Enhanced Virapower™ lentiviral vectors provide a highly efficient solution for gene delivery and expression in primary and other cell types which are refractory to more traditional gene delivery methods. Previously we demonstrated optimizations to the Virapower™ Lentiviral Expression vector line which included new HiPerform™ elements to increase the number of functional viral particle produced (functional titer) and a substantially boosted protein expression. Here we demonstrate how these enhancements allow for high levels of transduction efficiency and protein expression in primary neurons and other difficult cell types at high efficiency and low MOI. We also demonstrate the ability of HiPerform™ constructs to deliver and express native, functional membrane proteins including G-protein coupled receptors (GPCRs) in primary cells. The ability to produce active GPCRs, capable of antagonist ligand binding and displacement reactions, opens the door to creating novel tools for research and drug discovery and clinical applications.

Figure 1. The Virapower™ HiPerform™ Lentiviral Expression System Improvements

The ViraPower™ Lentiviral expression system offers a delivery tool applicable in a wide variety of cells. Lentivirus can target difficult to transfect cells including primary or non-dividing cells.

The new ViraPower™ HiPerform™ Lentiviral expression system includes the WPRE (Woodchuck post-transcriptional Regulatory Enhancer) and CPT (Polypurine Tract) sequences which enhance viral titer and expression in certain cell types. Lentiviral constructs containing the WPRE element exhibited a 5-8 fold increase in luciferase or GFP expression compared to lentivirus (1,2,3,5,6). A polypurine tract (CPT) from the HIV-1 integrase gene has been shown to increase the copy number of lentivirus integrating into the host genome (4).

The ViraPower™ HiPerform™ Lentiviral expression vectors offer a variety of options for titering and cloning. pLenti6.3 vectors feature blastidicin resistance for stringent titering and creation of stable cell lines. pLenti7.3 FastTiter™ vectors contain an EmGFP reporter in the vector backbone for rapid titering or high throughput cell sorting.

Virus is produced by co-transfecting the expression vector along with the ViraPower packaging mix containing genes required to construct non-replicating pseudoviral particles in 293FT cells. Virus is secreted into the growth medium and can be collected 48 hours post-transfection.

B. Titration:

Bed Protocol
FastTiter Protocol

- 4 weeks
- Stringent Titering
- Rapid titering
- Stable integrants
- High throughput screening

Diluted virus is dosed onto HT1080 cells. Successful transduction events express either blastidicin resistance (pLenti6.3) or GFP (pLenti7.3). Within 2 weeks, blastidicin resistant cells form foci which can be stained with crystal violet. In the case of cells transduced with pLenti7.3 FastTiter™ vectors, GFP fluorescence can be detected visually within 48 hours and productive transduction events can be quantitated on a flow cytometer.

A similar experiment performed with pLenti 6.3/V5-BGal showed negligible beta-galactosidase activity in transfected rat hippocampal neurons. Expression level of the enzyme could be controlled by regulating the viral dose.

The near-physiological levels of expression generated by Virapower™ lentivirus means transgenes can be expressed in cells in biological context. Here, two GPCR’s from Invitrogen’s Ultimate ORF Collection were cloned into Virapower™ HiPerform™ lentivirus. Transfections were optimized by titrating transfection reagent concentration. However, even at maximal efficiency, delivery was only 4%. In contrast, lentivirus delivered the GFP expression construct with high efficiency (80% efficiency at MOI=1). GFP expression (fluorescence intensity) was also substantially higher in transfected cells.

Rock hippocampal neurons (embryonic day 18) were plated at 80% confluency and either transfected with pLenti 6.3/V5-CFP or transduced with the same construct packaged into Virapower™ HiPerform™ lentivirus. Transfections were optimized by titrating transfection reagent concentration. However, even at maximal efficiency, delivery was negligible. In contrast, 30-60% transduction efficiency was obtained with a broad range of MOIs.

References